PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Application of: Ullrich *et al.*

Serial No.: 08/426,509 Group Art Unit: 1812

Filed: April 21, 1995 Examiner: Teng, S. P.

For: NOVEL MEGAKARYOCYTIC PROTEIN TYROSINE KINASES Attorney Docket No.: 7683-074-999

MT 12/4/96 #15

DECLARATION OF AXEL ULLRICH, MIKHAIL GISHIZKY, AND IRMIGARD SURES UNDER 37 CFR § 1.131

Assistant Commissioner for Patents
Washington, D.C. 20231

We, Axel Ullrich, Ph.D., who resides at Adalbertstr. 108, 80798 München, Germany, Mikhail Gishizky, Ph.D., who resides at 3001 B. Bryant Street, Palo Alto, California, and Irmigard Sures, Ph.D., who resides at Forstenrieder, Allee 55, 8000 München, Germany, do declare that:

1. We are the co-inventors of the presently claimed invention in the above-identified application.

2. The above-identified patent application relates to the isolation and characterization of novel non-receptor tyrosine kinase proteins from megakaryocytes, which are referred to as MKK1, MKK2, and MKK3. These proteins are characterized by an intracellular tyrosine kinase domain, a SH2 src homology domain, and a SH3 src homology domain.

3. Attached hereto are copies of the following Exhibits:

EXPRESS MAIL CERTIFICATION

"Express Mail" label No._____ Date of Deposit_____.
I hereby certify that this paper or fee is being deposited with the United States Postal Service "Express Mail Post Office to Addressee" service under 37 C.F.R. 1.10 on the date indicated above and is addressed to the Commissioner of Patents and Trademarks, Washington, D.C. 20231.

(A) Exhibit A is a copy of page 54 from laboratory notebook number 930014 of Brad Yatabe.

(D) Exhibit B is a copy of a page from laboratory notebook number 930021 of Brad Yatabe relating to the successful expression of MKK1 fusion proteins.

4. We have reviewed each of the documents of Exhibits A and B. Although the dates have been blanked out, each of these documents was dated prior to January 14, 1994. We hereby confirm that the work evidenced by each of the documents of Exhibits A and B, and all acts relied upon in these Exhibits for this declaration were carried out in the United States of America prior to January 14, 1994.

5. The experimental work reflected in Exhibits A and B was performed at our direction and supervision by Brad Yatabe, who was Mikhail Gishizky's research associate and an employee of SUGEN, Inc.

6. Prior to January 14, 1994, we conceived the idea of expressing MKK1 DNA to synthesize MKK peptides, fusion proteins and full length proteins. Our plan was to express MKK peptides and fusion proteins in order to generate antibodies that could be used to detect the successful expression of full length MKK1.

7. Prior to January 14, 1994, we directed the successful preparation of MKK1 fusion proteins. In particular, our experimental plan to produce fusion proteins of MKK1 with the glutathione-S-transferase protein (GST) is outlined in Exhibit A. MKK1 coding sequence is fused in frame with GST coding region in the bacterial expression plasmid pGEX-2T. The resulting expression plasmid is transformed into E. coli. Induction of transcription from the plasmid results in bacterial expression of fusion protein. These fusion proteins are denoted "MKK1/pGex(GST) proteins" at the top of this page. Below this heading is presented an "Outline" of the experimental steps to achieve this goal. A polynucleotide fragment of MKK1 coding sequence which includes the SH3 domain is indicated with the circled roman numeral "I". Fragment I extends from the EcoRI site upstream of the initiation codon "ATG" to a Bgl II site downstream of the SH3 coding region; hence, fragment "I" is also referred to as "NH_3 R1-Bgl 2 frag." at the right side of the page. Fragment "II" extends from the Sma I site within the coding region to the

Bgl II site. As indicated to the right of fragment II by the term "78 aa", this fragment encodes 78 amino acids of MKK1. Fragment III refers to a Sma I to Kpn I fragment. On the right side of the page is indicated that this fragment encodes "33 aa" (33 amino acids) of MKK1. Fragment II was subcloned as shown in frame with the GST coding sequence in the bacterial expression plasmid "pGex-2T" to produce the plasmid "pGexMKK1(8-85)."

6. Prior to January 14, 1994 we successfully expressed MKK1/GST fusion proteins and analyzed the resulting protein products by SDS-PAGE (SDS polyacrylamide gel electrophoresis). Specifically, bacteria were transformed with expression vectors including a "pGEX control" vector, and "MKK-1(10) & MKK-1(12) clones." The MKK-1(10) clone was transformed with the pGexMKK1(8-85) plasmid described in Exhibit A. This plasmid directed the expression of a fusion protein containing 78 amino acids of MKK1 (including most of the SH3 domain) fused in-frame to GST. Expression from this plasmid was induced by treatment of the bacterial culture with Isopropyl β-D-Thiogalactopyranoside, which is indicated as "1 mM IPTG". Several different uninduced and induced cultures were run side-by-side on a 12.5% PAGE gel. The marker lane contains molecular weight standards of 106, 80, 49.5, 32.3 and 29.2 kilodaltons. As indicated at the bottom of the PAGE gel, the MKK-1(10) bacterial clones were run in two lanes just to the right of the marker lane. The lane adjacent to the marker lane is an uninduced bacterial culture; the next lane is an induced (labeled "Ind") bacterial culture. Comparison of the uninduced and induced lanes indicates that treatment of the bacterial culture with IPTG successfully induced the production of a fusion protein which ran approximately midway between the 32.3 kd and 49.5 kd marker bands. The GST fusion protein produced by the MKK-1(10) clone was purified and used to generate polyclonal antibodies which recognized the 78 amino acids encoded by the Sma I to Bgl II fragment of the MKK1 gene, as described in the above captioned application at page 40, lines 25 to 31.

9. The antibodies raised against the GST fusion protein described in Exhibits A and B, comprising the 78 amino acids encoded by the Sma I to Bgl II fragment of the MKK1 gene fused to GST, were used to isolate MKK1 protein. As described in the above captioned application at page 40, line 34 to page 41, line 5, the coding region of

the MKK1 cDNA was cloned into the pBLUESCRIPT plasmid. Using this plasmid, the MKK1 coding sequence was transcribed and translated in the presence of ^{35}S-methionine using standard methods. Following protein synthesis, MKK1 protein was immunoprecipitated with the polyclonal antibodies raised against the GST fusion protein. Immunoprecipitated MKK1 was run on a PAGE gel; the results of this experiment are shown in Figure 5 of the instant specification.

10. Accordingly, prior to January 14, 1994, we conceived and reduced to practice to practice and/or conceived and were diligent in reducing to practice the production of MKK1 polypeptides as presently claimed.

11. We declare further that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of United States Code and that such willful false statements may jeopardize the validity of the application of any patent issuing thereon.

DATED:_____

Axel Ullrich

DATED:_____

Irmigard Sures

DATED: 11-1-96

Mikhail Gishizky

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DATED: 11/11/96 Axel Ullrich

DATED: 11/4/96 Irmigard Sures

DATED: 10-1-96 Mikhail Gishizky

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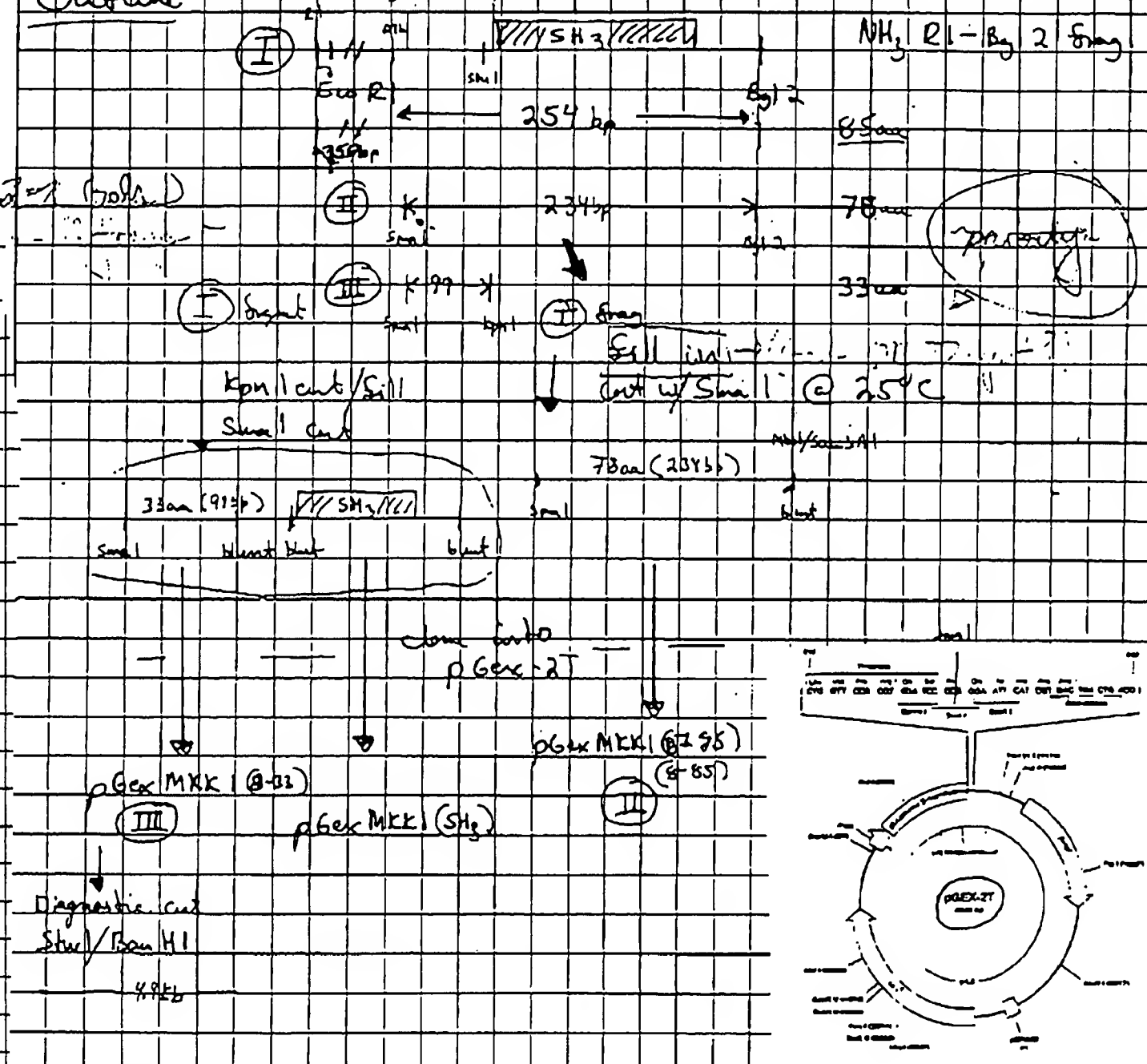
Notebook No. 90014 p3001

PROJECT

Continued From Page 2

Purpose: Make MKK1/pGex (GST) protein to be used for generating Ab

Outline:



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Read and Understood By

Signed Date Signed Date

Notebook No. 930021

PROJECT ____________

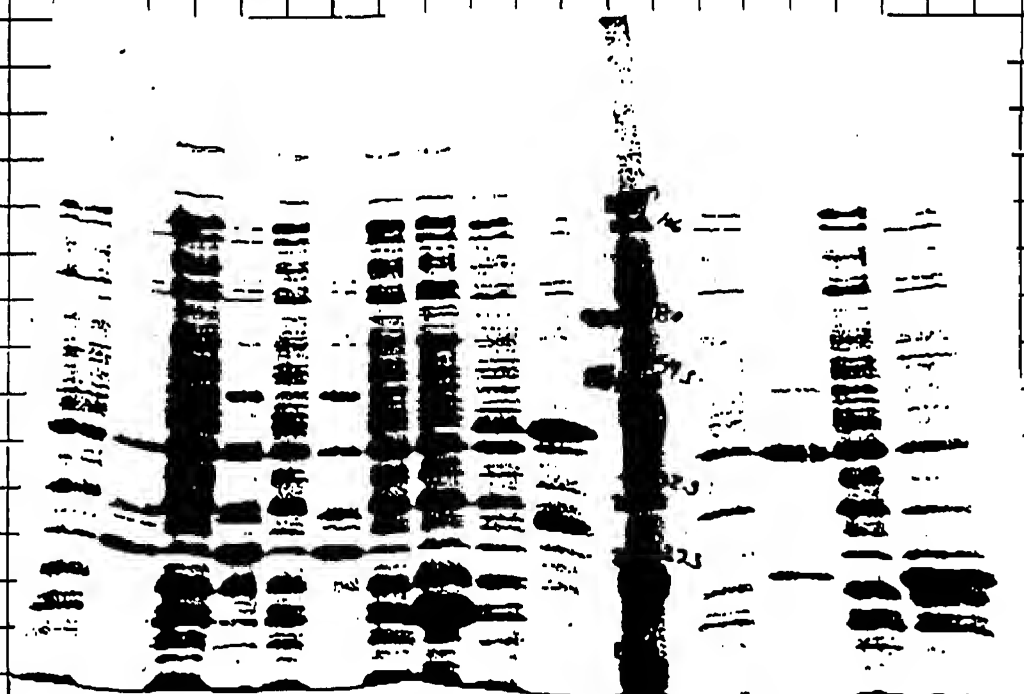
Continued From Page ______

B.

Purpose: Run PAGE of mKK-1 [illegible] samples to check for induction of protein, also run pGEX control and mKK-1(10) & mKK-1(12) clones.

Procedure

To help process, am inducing w/ 1mM IPTG instead of .1mM
and in processing samples am making sure samples are well homogenized
stain gels 4 hours or greater
run on 12.5% PAGE



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Read and Understood By

Signed: By Date ______ Signed: [signature] Date ______